SEQUENCE LISTING

<110> The Curators of the University of Missouri

<120> LARGE SCALE EXPRESSION AND PURIFICATION OF RECOMBINANT PROTEINS

<130> UMO1531.1

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<150> 60/218,125
<151> 2000-01-13

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<170> PatentIn Ver. 2.1

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<223> A Poly (H) affinity tag comprising 6 His residues have been inserted at the C-Terminus end of the coding region of the protein

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                               Met Leu Gln Ph  Ser Leu Ser Pro Thr
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Leu Ser Met Gly Phe His Val Ile Ala Met Val Ala Leu Leu Phe Ser
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cat gtg gac cat ata agt gct gag aca gaa atg gaa gga gaa ggc aac   390
His Val Asp His Ile Ser Ala Glu Thr Glu Met Glu Gly Glu Gly Asn
                 30                  35                  40

gag act ggc gag tgt act ggc tcc tat tac tgt aag aag ggg gtg att   438
Glu Thr Gly Glu Cys Thr Gly Ser Tyr Tyr Cys Lys Lys Gly Val Ile
             45                  50                  55

tta ccc att tgg gag ccc cag gac cct tcc ttt gga gac aaa att gct   486
Leu Pro Ile Trp Glu Pro Gln Asp Pro Ser Phe Gly Asp Lys Ile Ala
         60                  65                  70

aga gcg act gtg tat ttt gtg gcc atg gtc tac atg ttt ctt gga gtc   534
Arg Ala Thr Val Tyr Phe Val Ala Met Val Tyr Met Phe Leu Gly Val
     75                  80                  85

tca atc att gct gac cgg ttc atg tcc tct ata gaa gtc atc acg tct   582
Ser Ile Ile Ala Asp Arg Phe Met Ser Ser Ile Glu Val Ile Thr Ser
 90                  95                 100                 105

caa gag aaa gaa atc acc ata aag aaa ccc aat gga gag acc acc aag   630
Gln Glu Lys Glu Ile Thr Ile Lys Lys Pro Asn Gly Glu Thr Thr Lys
                110                 115                 120

aca act gtg agg atc tgg aat gag aca gtg tcc aac ctg acc ttg atg   678
Thr Thr Val Arg Ile Trp Asn Glu Thr Val Ser Asn Leu Thr Leu Met
            125                 130                 135

gcc ctg ggg tct tca gct cca gag att ctc ctt tca gta atc gag gtg   726
Ala Leu Gly Ser Ser Ala Pro Glu Ile Leu Leu Ser Val Ile Glu Val
        140                 145                 150

tgt ggc cat aac ttc act gca gga gac ctt ggc cct agc acc atc gtg   774
Cys Gly His Asn Phe Thr Ala Gly Asp Leu Gly Pro Ser Thr Ile Val
    155                 160                 165

ggg agt gct gca ttc aac atg ttc atc atc att gcc ctt tgt gtg tat   822
Gly Ser Ala Ala Phe Asn Met Phe Ile Ile Ile Ala Leu Cys Val Tyr
170                 175                 180                 185
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gtc gtc ccg gat ggg gag aca agg aag atc aag cat ctg cgt gtg ttc   870
Val Val Pro Asp Gly Glu Thr Arg Lys Ile Lys His Leu Arg Val Phe
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ttt gtg aca gca gca tgg agc atc ttt gcc tat acc tgg ctt tac atc   918
Phe Val Thr Ala Ala Trp Ser Ile Phe Ala Tyr Thr Trp Leu Tyr Ile
            205                 210                 215

att ttg tct gtc agc tcc cct ggg gtc gtg gag gtc tgg gaa ggt ttg   966
Ile Leu Ser Val Ser Ser Pro Gly Val Val Glu Val Trp Glu Gly Leu
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ctt act ttc ttc ttc ttc ccc atc tgc gtt gtg ttt gct tgg gtg gca   1014
Leu Thr Phe Phe Phe Phe Pro Ile Cys Val Val Phe Ala Trp Val Ala
    235                 240                 245

gac agg agg ctt ctg ttt tac aag tat gtc tac aag agg tat cgg gct   1062
Asp Arg Arg Leu Leu Phe Tyr Lys Tyr Val Tyr Lys Arg Tyr Arg Ala
250                 255                 260                 265

ggc aag cag agg gga atg att att gaa cac gaa gga gac agg cca tct   1110
Gly Lys Gln Arg Gly Met Ile Ile Glu His Glu Gly Asp Arg Pro Ser
                270                 275                 280

tcc aag aca gaa att gaa atg gat ggg aaa gtg gtc aat tcc cat gtt   1158
Ser Lys Thr Glu Ile Glu Met Asp Gly Lys Val Val Asn Ser His Val
            285                 290                 295

gac agt ttc tta gat gga gcc ctg gtt ctg gag gtt gat gag agg gac   1206
Asp Ser Phe Leu Asp Gly Ala Leu Val Leu Glu Val Asp Glu Arg Asp
        300                 305                 310

caa gat gat gaa gaa gcc agg cga gaa atg gct agg att ctg aag gaa   1254
Gln Asp Asp Glu Glu Ala Arg Arg Glu Met Ala Arg Ile Leu Lys Glu
    315                 320                 325

ctc aag cag aag cat cca gag aag gaa ata gag caa tta ata gaa tta   1302
Leu Lys Gln Lys His Pro Glu Lys Glu Ile Glu Gln Leu Ile Glu Leu
330                 335                 340                 345

gcc aat tac caa gtc tta agt cag cag caa aaa agt cga gcg ttt tac   1350
Ala Asn Tyr Gln Val Leu Ser Gln Gln Gln Lys Ser Arg Ala Phe Tyr
                350                 355                 360

cgt att caa gct acc cgc ctg atg acc gga gca ggc aac att tta aag   1398
Arg Ile Gln Ala Thr Arg Leu Met Thr Gly Ala Gly Asn Ile Leu Lys
            365                 370                 375
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agg cat gca gca gac caa gcc agg aaa gct gtc agc atg cat gag gtc   1446
Arg His Ala Ala Asp Gln Ala Arg Lys Ala Val Ser Met His Glu Val
        380                 385                 390

aac acg gaa gtg gct gaa aat gac cct gtc agt aag atc ttc ttt gaa   1494
Asn Thr Glu Val Ala Glu Asn Asp Pro Val Ser Lys Ile Phe Phe Glu
    395                 400                 405

caa ggg aca tat cag tgt ctg gag aac tgt ggc aca gta gcc ctg acc   1542
Gln Gly Thr Tyr Gln Cys Leu Glu Asn Cys Gly Thr Val Ala Leu Thr
410                 415                 420                 425

att atc cgc aga ggt ggt gat ttg acc aac act gtg ttt gtt gac ttc   1590
Ile Ile Arg Arg Gly Gly Asp Leu Thr Asn Thr Val Phe Val Asp Phe
                430                 435                 440

aga aca gag gat ggc aca gcc aat gct gga tct gat tac gaa ttt acc   1638
Arg Thr Glu Asp Gly Thr Ala Asn Ala Gly Ser Asp Tyr Glu Phe Thr
            445                 450                 455

gaa gga act gtg gtc ttt aag cct ggt gag acc cag aag gaa atc aga   1686
Glu Gly Thr Val Val Phe Lys Pro Gly Glu Thr Gln Lys Glu Ile Arg
        460                 465                 470

gtt ggc atc att gat gat gac atc ttt gag gag gat gag aat ttc ctt   1734
Val Gly Ile Ile Asp Asp Asp Ile Phe Glu Glu Asp Glu Asn Phe Leu
    475                 480                 485

gtg cat ctc agc aac gtc aaa gta tct ttg gaa gcc tcg gaa gac ggc   1782
Val His Leu Ser Asn Val Lys Val Ser Leu Glu Ala Ser Glu Asp Gly
490                 495                 500                 505

atc ctg gaa gcc agt cat gtc tct acc ctt gct tgc ctg gga tcc ccc   1830
Ile Leu Glu Ala Ser His Val Ser Thr Leu Ala Cys Leu Gly Ser Pro
                510                 515                 520

tcc act gcc acc gtg act att ttt gat gat gac cat gct ggc atc ttt   1878
Ser Thr Ala Thr Val Thr Ile Phe Asp Asp Asp His Ala Gly Ile Phe
            525                 530                 535

act ttt gag gaa ccg gtg act cat gtg agt gag agc att ggc atc atg   1926
Thr Phe Glu Glu Pro Val Thr His Val Ser Glu Ser Ile Gly Ile Met
        540                 545                 550

gag gtg aaa gtt ctg aga aca tct gga gca cgt gga aat gtt atc gtt   1974
Glu Val Lys Val Leu Arg Thr Ser Gly Ala Arg Gly Asn Val Ile Val
    555                 560                 565
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ccc tat aag acc att gag ggg acc gcc aga ggt gga ggg gag gac ttt   2022
Pro Tyr Lys Thr Ile Glu Gly Thr Ala Arg Gly Gly Gly Glu Asp Phe
570                 575                 580                 585

gag gac aca tgc gga gag ctc gag ttc cag aat gac gaa att gtc aaa   2070
Glu Asp Thr Cys Gly Glu Leu Glu Phe Gln Asn Asp Glu Ile Val Lys
                590                 595                 600

aca ata tca gtc aag gta att gat gat gag gag tat gag aaa aac aag   2118
Thr Ile Ser Val Lys Val Ile Asp Asp Glu Glu Tyr Glu Lys Asn Lys
            605                 610                 615

acc ttc ttc ctt gag att gga gag ccc cgc ctg gtg gag atg agt gag   2166
Thr Phe Phe Leu Glu Ile Gly Glu Pro Arg Leu Val Glu Met Ser Glu
        620                 625                 630

aag aaa gcc ctg tta ttg aat gag ctt ggt ggc ttc aca ata aca ggg   2214
Lys Lys Ala Leu Leu Leu Asn Glu Leu Gly Gly Phe Thr Ile Thr Gly
    635                 640                 645

aaa tac ctg tat ggc cag cct gtc ttc agg aaa gtt cat gct aga gaa   2262
Lys Tyr Leu Tyr Gly Gln Pro Val Phe Arg Lys Val His Ala Arg Glu
650                 655                 660                 665

cat cca ctc ccc tct act ata atc acc atc gca gat gaa tat gat gac   2310
His Pro Leu Pro Ser Thr Ile Ile Thr Ile Ala Asp Glu Tyr Asp Asp
                670                 675                 680

aag cag cca ctg acc agc aaa gag gag gaa gag agg cgc att gcg gaa   2358
Lys Gln Pro Leu Thr Ser Lys Glu Glu Glu Glu Arg Arg Ile Ala Glu
            685                 690                 695

atg ggg cgc ccc att ctg gga gag cac acc aga ctg gag gtg atc att   2406
Met Gly Arg Pro Ile Leu Gly Glu His Thr Arg Leu Glu Val Ile Ile
        700                 705                 710

gaa gaa tcc tac gag ttc aag agt acc gtg gac aaa ctg att aag aag   2454
Glu Glu Ser Tyr Glu Phe Lys Ser Thr Val Asp Lys Leu Ile Lys Lys
    715                 720                 725

aca aac cta gcc ctc gtg gtt ggg acg aac agc tgg aga gag cag ttc   2502
Thr Asn Leu Ala Leu Val Val Gly Thr Asn Ser Trp Arg Glu Gln Phe
730                 735                 740                 745

atc gag gcg atc act gtc agt gct ggg gaa gat gac gat gac gac gaa   2550
Ile Glu Ala Ile Thr Val Ser Ala Gly Glu Asp Asp Asp Asp Asp Glu
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tgt ggg gag gag aag ctg ccc tcc tgt ttt gac tac gtg atg cac ttt   2598
Cys Gly Glu Glu Lys Leu Pro Ser Cys Phe Asp Tyr Val Met His Ph
            765                 770                 775

ctg act gtg ttc tgg aag gtc ctc ttc gcc ttt gtc ccc ccg aca gag   2646
Leu Thr Val Phe Trp Lys Val Leu Phe Ala Phe Val Pro Pro Thr Glu
        780                 785                 790

tac tgg aac ggc tgg gcg tgt ttc atc gtc tcc atc ctc atg atc ggc   2694
Tyr Trp Asn Gly Trp Ala Cys Phe Ile Val Ser Ile Leu Met Ile Gly
    795                 800                 805

cta ctg acg gct ttc att gga gac ctc gct tcc cac ttc gcc tgc acc   2742
Leu Leu Thr Ala Phe Ile Gly Asp Leu Ala Ser His Phe Ala Cys Thr
810                 815                 820                 825

atc gcc ctg aag gat tcc gtg acc gcg gtg gtg ttc gtt gcg ctt gga   2790
Ile Ala Leu Lys Asp Ser Val Thr Ala Val Val Phe Val Ala Leu Gly
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acc tca gtg cca gac aca ttt gca agc aaa gtg gcc gcc acc cag gac   2838
Thr Ser Val Pro Asp Thr Phe Ala Ser Lys Val Ala Ala Thr Gln Asp
            845                 850                 855

cag tat gcg gat gca tcc ata ggt aac gtc aca ggc agc aac gcg gtg   2886
Gln Tyr Ala Asp Ala Ser Ile Gly Asn Val Thr Gly Ser Asn Ala Val
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aac gtc ttc ctg ggc atc ggt gtg gcc tgg tcc atc gcc gcc atc tac   2934
Asn Val Phe Leu Gly Ile Gly Val Ala Trp Ser Ile Ala Ala Ile Tyr
    875                 880                 885

cac gcg gcc aac ggg gaa cag ttc aaa gtg tcc cct ggc acg cta gct   2982
His Ala Ala Asn Gly Glu Gln Phe Lys Val Ser Pro Gly Thr Leu Ala
890                 895                 900                 905

ttt tct gtc act ctc ttc acc att ttt gct ttc atc aat gtg ggg gtg   3030
Phe Ser Val Thr Leu Phe Thr Ile Phe Ala Phe Ile Asn Val Gly Val
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ctg ctg tat cgg cgg agg cca gaa att gga ggt gag ctg ggt ggg ccc   3078
Leu Leu Tyr Arg Arg Arg Pro Glu Ile Gly Gly Glu Leu Gly Gly Pro
            925                 930                 935

cgg act gcc aag ctc ctc aca tcc tgc ctc ttt gtg ctc ctg tgg ctc   3126
Arg Thr Ala Lys Leu Leu Thr Ser Cys Leu Phe Val Leu Leu Trp L u
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ttg tac att ttc ttc tcc tcc ctg gag gcc tac tgc cac ata aaa ggc 3174
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955 960 965

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Phe
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cataaaaatt atgtataatg aacagaggaa actggcattt gtcatgtcca cccacctgct 3290

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Asp Pro Ser Phe Gly Asp Lys Ile Ala Arg Ala Thr Val Tyr Phe Val
 65                  70                  75                  80
Ala Met Val Tyr Met Phe Leu Gly Val Ser Ile Ile Ala Asp Arg Phe
                 85                  90                  95
Met Ser Ser Ile Glu Val Ile Thr Ser Gln Glu Lys Glu Ile Thr Ile
            100                 105                 110
Lys Lys Pro Asn Gly Glu Thr Thr Lys Thr Thr Val Arg Ile Trp Asn
        115                 120                 125
Glu Thr Val Ser Asn Leu Thr Leu Met Ala Leu Gly Ser Ser Ala Pro
    130                 135                 140
Glu Ile Leu Leu Ser Val Ile Glu Val Cys Gly His Asn Phe Thr Ala
145                 150                 155                 160
Gly Asp Leu Gly Pro Ser Thr Ile Val Gly Ser Ala Ala Phe Asn Met
                165                 170                 175
Phe Ile Ile Ile Ala Leu Cys Val Tyr Val Val Pro Asp Gly Glu Thr
            180                 185                 190
Arg Lys Ile Lys His Leu Arg Val Phe Phe Val Thr Ala Ala Trp Ser
        195                 200                 205
Ile Phe Ala Tyr Thr Trp Leu Tyr Ile Ile Leu Ser Val Ser Ser Pro
    210                 215                 220
Gly Val Val Glu Val Trp Glu Gly Leu Leu Thr Phe Phe Phe Phe Pro
225                 230                 235                 240
Ile Cys Val Val Phe Ala Trp Val Ala Asp Arg Arg Leu Leu Phe Tyr
                245                 250                 255
Lys Tyr Val Tyr Lys Arg Tyr Arg Ala Gly Lys Gln Arg Gly Met Ile
            260                 265                 270
Ile Glu His Glu Gly Asp Arg Pro Ser Ser Lys Thr Glu Ile Glu Met
        275                 280                 285
Asp Gly Lys Val Val Asn Ser His Val Asp Ser Phe Leu Asp Gly Ala
    290                 295                 300
Leu Val Leu Glu Val Asp Glu Arg Asp Gln Asp Asp Glu Glu Ala Arg
305                 310                 315                 320
Arg Glu Met Ala Arg Ile Leu Lys Glu Leu Lys Gln Lys His Pro Glu
                325                 330                 335
Lys Glu Ile Glu Gln Leu Ile Glu Leu Ala Asn Tyr Gln Val Leu Ser
            340                 345                 350
Gln Gln Gln Lys Ser Arg Ala Phe Tyr Arg Ile Gln Ala Thr Arg Leu
        355                 360                 365
Met Thr Gly Ala Gly Asn Ile Leu Lys Arg His Ala Ala Asp Gln Ala
    370                 375                 380
Arg Lys Ala Val Ser Met His Glu Val Asn Thr Glu Val Ala Glu Asn
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Asp Pro Val Ser Lys Ile Phe Phe Glu Gln Gly Thr Tyr Gln Cys Leu
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420 425 430

Leu Thr Asn Thr Val Phe Val Asp Phe Arg Thr Glu Asp Gly Thr Ala

435 440 445

Asn Ala Gly Ser Asp Tyr Glu Phe Thr Glu Gly Thr Val Val Phe Lys

450 455 460

Pro Gly Glu Thr Gln Lys Glu Ile Arg Val Gly Ile Ile Asp Asp Asp

465 470 475 480

Ile Phe Glu Glu Asp Glu Asn Phe Leu Val His Leu Ser Asn Val Lys

485 490 495

Val Ser Leu Glu Ala Ser Glu Asp Gly Ile Leu Glu Ala Ser His Val

500 505 510

Ser Thr Leu Ala Cys Leu Gly Ser Pro Ser Thr Ala Thr Val Thr Ile

515 520 525

Phe Asp Asp Asp His Ala Gly Ile Phe Thr Phe Glu Glu Pro Val Thr

530 535 540

His Val Ser Glu Ser Ile Gly Ile Met Glu Val Lys Val Leu Arg Thr

545 550 555 560

Ser Gly Ala Arg Gly Asn Val Ile Val Pro Tyr Lys Thr Ile Glu Gly

565 570 575

Thr Ala Arg Gly Gly Gly Glu Asp Phe Glu Asp Thr Cys Gly Glu Leu

580 585 590

Glu Phe Gln Asn Asp Glu Ile Val Lys Thr Ile Ser Val Lys Val Ile

595 600 605

Asp Asp Glu Glu Tyr Glu Lys Asn Lys Thr Phe Phe Leu Glu Ile Gly

610 615 620

Glu Pro Arg Leu Val Glu Met Ser Glu Lys Lys Ala Leu Leu Leu Asn

625 630 635 640

Glu Leu Gly Gly Phe Thr Ile Thr Gly Lys Tyr Leu Tyr Gly Gln Pro

645 650 655

Val Phe Arg Lys Val His Ala Arg Glu His Pro Leu Pro Ser Thr Ile

660 665 670

Ile Thr Ile Ala Asp Glu Tyr Asp Asp Lys Gln Pro Leu Thr Ser Lys

675 680 685

Glu Glu Glu Glu Arg Arg Ile Ala Glu Met Gly Arg Pro Ile Leu Gly

690 695 700

Glu His Thr Arg Leu Glu Val Ile Ile Glu Glu Ser Tyr Glu Phe Lys

705 710 715 720

Ser Thr Val Asp Lys Leu Ile Lys Lys Thr Asn Leu Ala Leu Val Val

725 730 735

Gly Thr Asn Ser Trp Arg Glu Gln Phe Ile Glu Ala Ile Thr Val Ser

740 745 750

Ala Gly Glu Asp Asp Asp Asp Asp Glu Cys Gly Glu Glu Lys Leu Pro

755 760 765

Ser Cys Phe Asp Tyr Val Met His Phe Leu Thr Val Phe Trp Lys Val

770 775 780

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785 790 795 800

Ph Ile Val Ser Ile Leu Met Ile Gly Leu Leu Thr Ala Phe Ile Gly
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Ala Ser Lys Val Ala Ala Thr Gln Asp Gln Tyr Ala Asp Ala Ser Ile
850 855 860

Gly Asn Val Thr Gly Ser Asn Ala Val Asn Val Phe Leu Gly Ile Gly
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Phe Lys Val Ser Pro Gly Thr Leu Ala Phe Ser Val Thr Leu Phe Thr
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Ile Phe Ala Phe Ile Asn Val Gly Val Leu Leu Tyr Arg Arg Arg Pro
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Glu Ile Gly Gly Glu Leu Gly Gly Pro Arg Thr Ala Lys Leu Leu Thr
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945 950 955 960

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